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Detection of White-footed Deermouse (*Peromyscus leucopus*) on Grand Manan Island, New Brunswick, Canada: Taxonomic, Biogeographic, and Human-health Implications

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Abstract

Grand Manan Island represents the southernmost region of the province of New Brunswick, Canada. Due to its isolation, the island fauna is depauperate, with few native mammal species. Recent molecular genetic analysis of *Peromyscus* collected from GMI revealed the mitotype (cytochrome *b* gene) indicative of *P. leucopus* (White-footed Deermouse). This species previously was known only from mainland Nova Scotia in Maritime Canada. Here we document the occurrence of *P. leucopus* on Grand Manan Island and discuss the implications of this discovery, most notably as it relates to the endemic *P. maniculatus argentatus* (Grand Manan Deermouse) and the presence of Lyme disease.

Key words: Canada, Grand Manan Island, Lyme disease, *Peromyscus leucopus*, *Peromyscus maniculatus argentatus*

Introduction

Grand Manan Island (GMI) is located approximately 25 km off the south coast of New Brunswick, Canada in the Bay of Fundy. The terrestrial mammal fauna of GMI is relatively depauperate because it is isolated by cold waters (average summer high of 8–12° C), strong currents, and dramatic tidal fluctuations. Only nine terrestrial mammal species are considered native to the island; others were introduced via anthropogenic activity [e.g., *Rattus norvegicus* (Berkenhout, 1769), Norway Rat; *Mus musculus* (Linnaeus, 1758), House Mouse; *Tamiasciurus hudsonicus* (Erxleben, 1777), American Red Squirrel; *Ondatra zibithecus* (Linnaeus, 1766), Muskrat; *Odocoileus virginianus* (Zimmermann, 1780), White-tailed Deer] (Copeland and Church 1906; Ingersoll and Gorham 1978).

During the course of a small mammal field program focused on the GMI *Peromyscus maniculatus argentatus* (Deermouse) (Copeland and Church 1906), molecular genetic analysis detected *P. leucopus* (Rafinesque, 1818; White-footed Deermouse) on the island, a species previously not recorded from New Brunswick. *Peromyscus leucopus* is a congener of, but not sister to, *P. maniculatus* (Wagner, 1845; see Bradley et al.

2007, Bradley et al. 2019), and generally has a more southern distribution (Hall 1981; Lackey et al. 1985). *Peromyscus leucopus* in Maritime Canada was previously understood to be confined to mainland Nova Scotia, although the species also is present on islands off the coast of Maine, USA, the closest being Great Wass Island about 56 km southwest of GMI (Rich 1993; Naughton 2012). Here we document the recent discovery of *P. leucopus* on GMI. We also comment on the implications for our understanding of the subspecific status of *P. maniculatus argentatus* (endemic to GMI) and the need for a better understanding of both *Peromyscus* species on GMI given the high prevalence of Lyme disease on GMI.

MATERIALS AND METHODS

Trapping protocols followed guidelines set by the Animal Care Committee of the American Society of Mammalogists (Gannon et al. 2007). *Peromyscus* (n = 13) were collected from four localities on Grand Manan Island (Fig. 1) in July 2011 using museum special snap traps baited with a mixture of oats and peanut butter. Among those localities was Grand Harbor (44°41'26"N, 66°46'15"W), the type locality for the Deermouse subspecies originally described as *P. canadensis argentatus* by Copeland and Church (1906) (although the common name was noted as "Grand Manan White-footed Mouse"), and then revised to *P. maniculatus argentatus* by Osgood (1909). *Peromyscus maniculatus* (n = 22) also were collected from Jacquet River Gorge Protected Natural Area in northern mainland New Brunswick. Heart, liver, and muscle tissues were sampled, and vouchers consisting of skins and full skeletons were prepared and deposited in the New Brunswick Museum (NBM). Tissue biopsies were stored temporarily in 95% ethanol and then archived at -80ºC in the NBM frozen tissue collections.

Genomic DNA was extracted from subsamples of tissues at Texas Tech University using a QIAGEN Blood and Tissue Kit; the DNA subsequently were stored in QIAGEN EB (Tris) buffer and archived at -40º C. The entire cytochrome *b* gene (1,143 base pairs) for almost all specimens was amplified via polymerase chain reaction (PCR) using primers MVZ05 (Smith and Patton 1993) and PERO3' (Tiemann-Boege et al. 2000). Thermal profiles for PCR consisted of the following: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 51°C for 1 min, and extension at 72°C for 2 min, with a final extension at 72°C for 7 min. PCR products were viewed on a 1% agarose gel. Most PCR products were purified using QIAGEN Gel Extraction and PCR Purification kits; some were purified with ExoSAP-IT (Affymetrix, Santa Clara, California).

Purified PCR products underwent cycle sequencing reactions using the aforementioned PCR, other internal primers (400F, 400R, and 700H – Peppers and Bradley 2000; Tiemann-Boege et al. 2000), and ABI Prism Big Dye version 3.1 Terminator technology (Applied Biosystems, Foster City, California). The subsequent products were purified using isopropanol cleanup protocols (SOP in Bradley Lab) and sequenced with an ABI 3100-Avant automated sequencer. Resulting sequences were aligned and proofed using Sequencher 4.10 software (Gene Codes, Ann Arbor, Michigan); chromatograms were examined to verify all base changes. Sequences were deposited in GenBank with the following accession numbers: MT156535–MT156569.

Sequence alignment and phylogenetic analyses were performed using MEGA6 (Tamura et al. 2013). Sequences were aligned with the ClustalW algorithm (Thompson et al. 1994). Model selection for inferring evolutionary history resulted in the selection of the HKY+G model (Hasegawa et al. 1985) (BIC = 3206.428, AIC = 2605.027, +G = 0.15). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach (Deng and Moore 2009). The phylogenetic tree with the highest log likelihood value (-1148.350) was chosen. Bootstrap analysis (Felsenstein 1985) (1,000 iterations) was used to evaluate nodal support. Kimura 2-parameter (K2P) genetic distance values (Kimura 1980) were estimated to compare rates of genetic divergence among sample groups. The K2P model was chosen for its utility as a

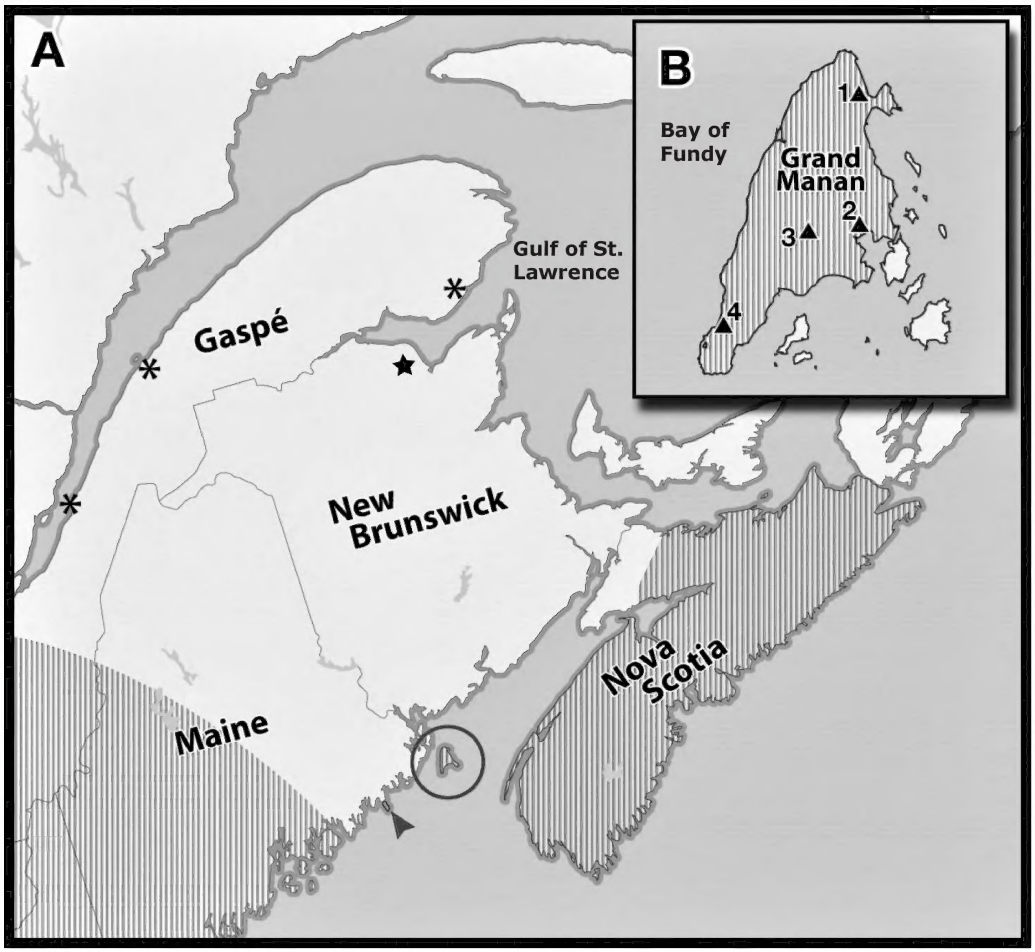

Figure 1. Distribution (cross-lines) of the White-footed Deermouse (*Peromyscus leucopus*) in Atlantic Canada and northeastern Maine (A; after Naughton 2012). Numbered triangles mark sites where the species was recorded on Grand Manan Island (B; inset) in 2011: 1. Rocky Corner, Whitehead Road, north (44°41'09"N, 66°47'27"W); 2. Grand Harbour (44°41'26"N, 66°46'15"W; also type locality for *P. maniculatus argentatus*); 3. Old dump site on Whitehead Road, south (44°47'18"N, 66°41'18"W); and 4. Bradford Cove (44°37'45"N, 66°53'26"W). Arrowhead marks Great Wass Island, Maine, USA. The star represents the locality (Jacquet River Gorge Protected Natural Area) where the Deermouse (*P. maniculatus*), were collected in northern New Brunswick. Asterisks denote sites in Gaspe, Quebec, where *P. leucopus* is found based on preliminary genetic data reported in Desroisiers et al. (2002).

distance metric in prior studies of rodent phylogenetics (Bradley and Baker 2001). Complete cytochrome *b* DNA sequences for *P. maniculatus* and *P. leucopus* from other localities were downloaded from GenBank (accession numbers DQ385784 and DQ000483, respectively) and added to the phylogenetic analysis as reference samples. *Peromyscus boylii* (Baird, 1855; Brush Deermouse) was used as the outgroup (GenBank accession number DQ000478).

RESULTS

Examination of the complete cytochrome *b* genetic sequences of *P. maniculatus* collected from GMI revealed several unexpected results. When these sequences were input into BLAST on GenBank, the mitotypes of these anomalous sequences matched those of *P. leucopus*, not *P. maniculatus*, *sensu lato*. Of the thirteen putatively identified *P. maniculatus* collected from GMI in 2011, nine (~ 69%) individuals manifested mitotypes that identified as *P. leucopus*. Four of these *P. leucopus* were collected at Grand Harbour, the type locality for *P. m. argentatus*.

Phylogenetically, two monophyletic clades were recovered: one clade included all *P. maniculatus*, and the other included all sequences of *P. leucopus* (Fig. 2). Estimated genetic distance (K2P = 9.47%) between *P. maniculatus* and *P. leucopus* groups confirmed the magnitude of genetic difference between the two clades. These specimens of *P. leucopus* from GMI represent the first records of this species for New Brunswick. Sequences of *P. maniculatus* from mainland New Brunswick showed little genetic differentiation (K2P = 1.12%) when compared to those from GMI.

DISCUSSION

There are several biogeographic implications that follow from the discovery of *P. leucopus* on GMI. This species may have been present on GMI since the Holocene Climatic Optimum, aka the Hypsithermal, a warm period that occurred ~9,000 to 4,000 BP (Pielou 1991). This species is hypothesized to be one member of a suite of species (see Petersen and Stewart 2006, Wisheu et al. 1994) that ranged through Maine and southern New Brunswick into Nova Scotia during the hypsithermal period (~6,000–4,000 BP). Subsequent climate cooling has now isolated these species (many of which are referred to as Atlantic coastal plain species; see Clayden et al. 2010) in mainland Nova Scotia, particularly the southwest. Indeed, several mammalian taxa exhibit this pattern of geographic disjunctiveness in Maritime Canada (e.g., see Broders et al. 2009, Petersen and Stewart 2006). In contrast, several studies have predicted and documented northward expansion of the geographic range of *P. leucopus* (e.g., Leo and Millien 2017, Roy-Dufresne et al. 2013), possibly facilitated by climate change. Although there is evidence that some Atlantic coastal plain species are beginning to respond to warming climate and expanding their ranges northward (e.g., see McAlpine et al. 2017), it is unclear if *P. leucopus* on GMI conforms to this pattern, especially given its presence already in Nova Scotia and (putatively) in the Gaspe region of Quebec. We note that Rich (1993) collected *Peromyscus* on GMI from 1989–1991, securing five specimens from Long Pond, all of which were identified as *P. maniculatus* based on electrophoretic analysis. However, this limited collection could well have missed *P. leucopus*, even if present.

The difficulty of natural dispersal to GMI for a small terrestrial mammal, and the lack of records for the species from adjacent mainland Maine and New Brunswick, suggest that *P. leucopus* has been present on GMI for some time but possibly was overlooked until molecular genetic identification methods were applied. *Peromyscus leucopus* is notoriously difficult to separate from *P. maniculatus* by morphological means alone (Choate 1973; Aquadro and Patton 1980; Feldhammer et al. 1983; Bruseo et al. 1999), even for sympatric regional populations (cf. see Rich et al. 1996 and Stephens et al. 2014). For example, Rich (1993) employed and tested morphological methods for separating the two *Peromyscus* species collected in New England and Nova Scotia but concluded that morphological identification of the two congeners was unreliable. [Cf., Kilpatrick et al. (1994) used genetic methods to positively identify *P. leucopus* on several islands in the Gulf of Maine.] This raises the prospect that the type series for *P. m. argentatus* may include individuals of both *P. maniculatus* and *P. leucopus*, and the original description for the subspecies may be a composite of the two species. Given that previous taxonomic authorities have continued to recognize *P. m. argentatus* as a distinct subspecies (e.g., Wilson and Reeder 2005, Bradley et al. 2017) but no comprehensive studies have been conducted to validate the original description and designation, the latest evidence presented here warrants such a taxonomic review.

Conversely, if *P. leucopus* is not a hypsithermal relict, then how did this species become established

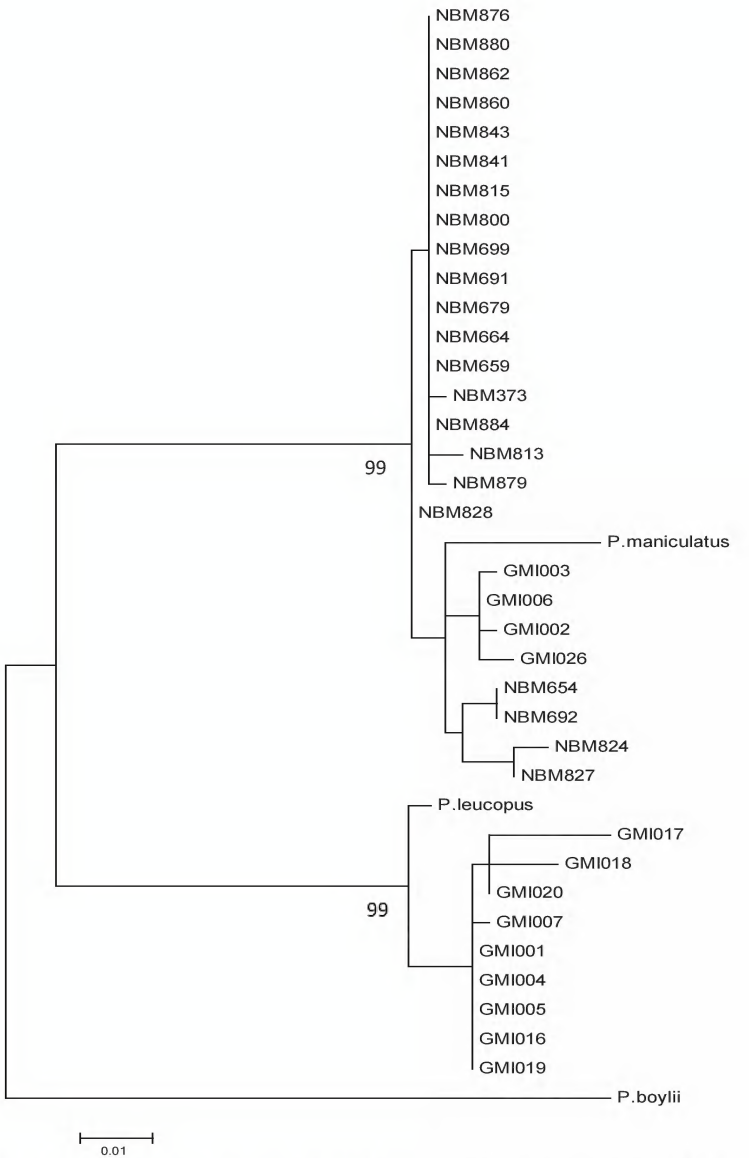

Figure 2. Maximum Likelihood phylogenetic tree depicting *Peromyscus* ($n = 35$) from mainland New Brunswick (NB) and Grand Manan Island (GMI). Numbers beside locality acronyms in the labels represent New Brunswick Museum field numbers. Bootstrap (1,000 iterations) values are shown at the nodes (>95% only). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Brush Deermouse (*Peromyscus boylii*) was used as the outgroup for the phylogenetic analysis; sequences of Deermouse (*P. maniculatus*) and White-footed Deermouse (*P. leucopus*) from GenBank were used for reference. Evolutionary analyses were conducted in MEGA6.

on GMI and when? Although we currently are unable to address this question definitively, arrival by natural (e.g., rafting or island hopping) and (or) anthropogenic means are both possible. Crowell (1986) identified some insular small mammal populations on islands in the Gulf of Maine as relictual but surmised that the population establishment of most species were dependent on recurrent colonization via ice bridges, a means of colonization unavailable for GMI taxa. Aquadro et al. (1980) suggested that establishment of *P. leucopus* on Mount Desert Island in Maine is relatively recent (i.e., post 1947) and the result of an increase in deciduous forest following wildfires. Although acknowledging that some might be relict populations, presence of *P. leucopus* generally was attributed to a range expansion underway for the species since the 1950s. Rich (1993) found a significant correlation between genetic distance of *Peromyscus* in the region and ocean depth between landmasses. This suggests limited gene flow among island and mainland populations of *Peromyscus* in the region and supports a post-Wisconsin relict status for at least those populations on the most isolated islands, including GMI.

An alternative explanation for the presence of *P. leucopus* on GMI is human-sponsored introduction. Human transportation between GMI and the New Brunswick mainland (Blacks Harbor) is readily accessible via daily ferries. It is also worth noting that hay was at one time exported to GMI from mainland New Brunswick to support agricultural activity (Hogg 2007), thereby providing a convenient vector of introduction for *Peromyscus*. However, it is not yet known whether human activity provides a vector for gene flow between the (current) mainland and the island populations of *Peromyscus* (though we detected little genetic differentiation among mainland and GMI *P. maniculatus*, our sample size for the GMI population was relatively small and we cannot make any conclusions). Regardless of whether colonization of GMI by *P. leucopus* has been natural or anthropogenic, and if the former, is relictual or recent, it could indicate the presence of adjacent mainland populations of this species in New Brunswick. Such populations have not been detected to date, but genetic investigations of adjacent mainland *Peromyscus* has been limited. The putative detection via genetic means of *P. leucopus* on the Gaspe (Desrosiers et al. 2002), well outside the generally accepted distributional range of the species, emphasizes the need for more detailed examination and study of *Peromyscus* across Atlantic Canada. In this study, use of molecular genetic markers unveiled the identity and presence of *P. leucopus* on GMI; strict reliance on morphological characters can easily lead to misidentifications, as some studies have reported. Indeed, previous biological surveys in New Brunswick may not have uncovered the presence of *P. leucopus* in New Brunswick because molecular genetic techniques either were not readily available and (or) were not implemented at the time.

More detailed studies of *Peromyscus* in Atlantic Canada would address more than just accurate taxonomy and biodiversity conservation. GMI has been categorized as a "hot-spot" for Lyme disease (http://www2.gnb.ca/content/gnb/en/departments/ocmoh/cdc/content/vectorborne_andzoonotic/Tick-Borne_Diseases/brief.html) by Health Canada. Lyme disease is caused by the bacteria spirochete *Borellia burgdorferi* and is spread by ticks (*Ixodes* sp.). Lyme disease management is quite complex with respect to understanding the interplay of various tick-pathogen-host dynamics, as several species of small mammals are known to be competent hosts for *B. burdorferi* (Bernard et al. 2020). Although both species of *Peromyscus* are competent reservoirs for Lyme disease (Lane et al. 1991), there is some evidence to suggest that *P. leucopus* may be the preferred and more competent host reservoir of the two (Donahue et al. 1987; Garman et al. 1994;V. Millien, pers. comm.). If so, the presence of *P. leucopus* as a host vector may increase the chances of transmission of *B. burgdorferi* to prospective human hosts [e.g., Ostfeld (2011) reports that *P. leucopus* often show a 75–95% efficiency of transmission]. How these two species of *Peromyscus* are interacting both zoonotically, with respect to Lyme disease epidemiology, and ecologically on GMI is not currently known. Garman et al. (1994), working on Mount Desert Island, found no evidence of negative interspecific interactions between the two species, although they noted that *P. leucopus* showed a distinct preference for deciduous habitat; to our knowledge, no ecological studies of *Peromyscus* have been conducted on GMI. Future work should focus on advancing knowledge of the distribution (with documentation in the form of vouchers and sequence data), relative abundance, and dynamics of species interactions for both *Peromyscus* species on GMI, particularly relative to the impacts of anthropogenic climate change and land use (e.g., effects caused by

deforestation). These efforts would be beneficial in terms of better understanding the prevalence and spread of Lyme disease on GMI (and in Atlantic Canada more generally) and informing wildlife management plans with respect to human health.

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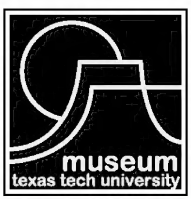

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